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# Translation Initiation on Mammalian mRNAs with Structured 5′UTRs Requires DExH-Box Protein DHX29

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## SUMMARY

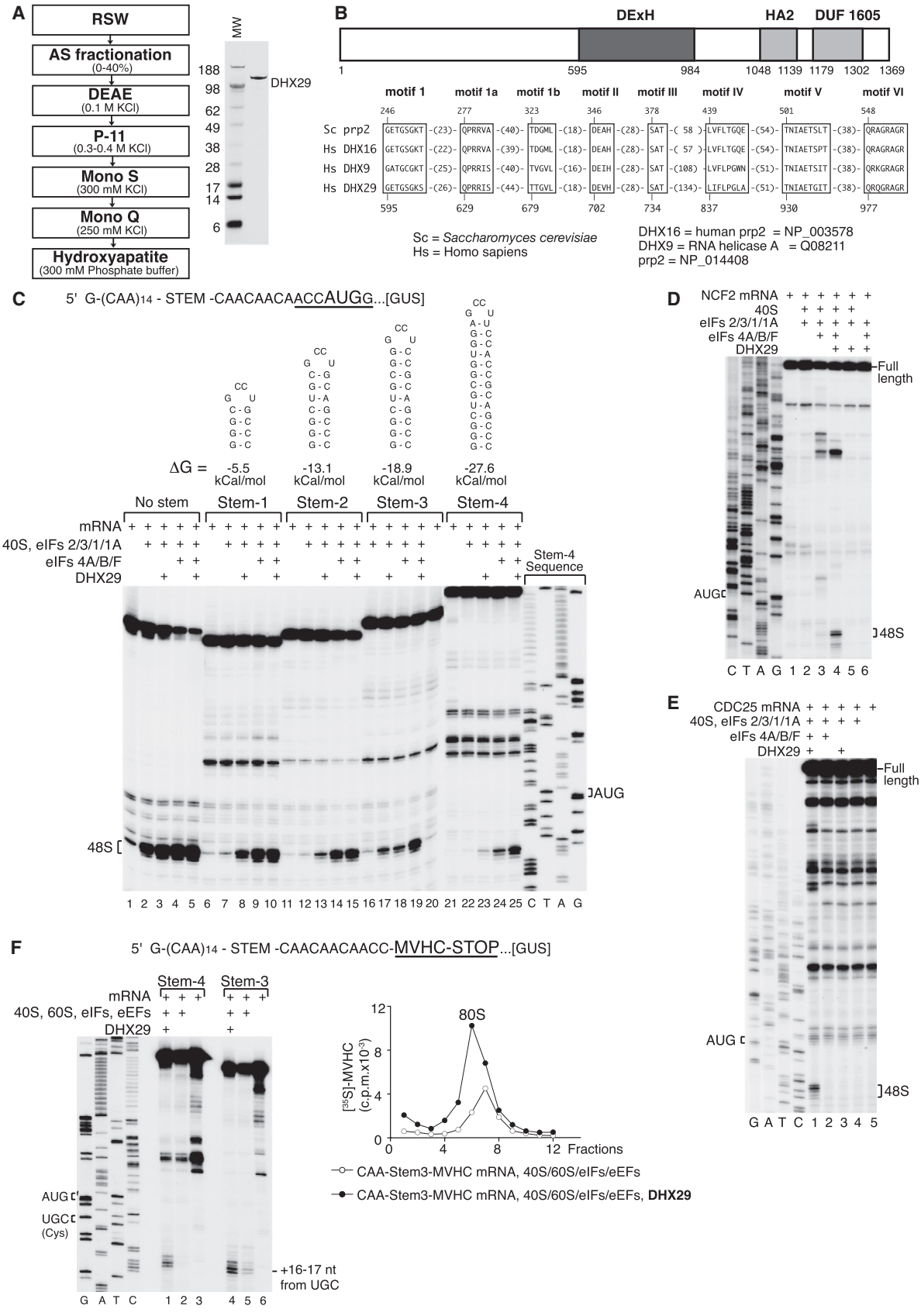
Eukaryotic protein synthesis begins with assembly of 48S initiation complexes at the initiation codon of mRNA, which requires at least seven initiation factors (eIFs). First, 43S preinitiation complexes comprising 40S ribosomal subunits, eIFs 3, 2, 1, and 1A, and $tRNA^{Met}_i$ attach to the 5′-proximal region of mRNA and then scan along the 5′ untranslated region (5′UTR) to the initiation codon. Attachment of 43S complexes is mediated by three other eIFs, 4F, 4A, and 4B, which cooperatively unwind the cap-proximal region of mRNA and later also assist 43S complexes during scanning. We now report that these seven eIFs are not sufficient for efficient 48S complex formation on mRNAs with highly structured 5′UTRs, and that this process requires the DExH-box protein DHX29. DHX29 binds 40S subunits and hydrolyzes ATP, GTP, UTP, and CTP. NTP hydrolysis by DHX29 is strongly stimulated by 43S complexes and is required for DHX29's activity in promoting 48S complex formation.

## INTRODUCTION

Eukaryotic protein synthesis begins with assembly of 48S initiation complexes, in which initiator tRNA (Met-$tRNA^{Met}_i$) is base-paired with the initiation codon of mRNA in the P site of the 40S subunit. 48S complex formation on most cellular mRNAs occurs by the scanning mechanism and requires at least seven initiation factors (eIFs) (Pestova et al., 2007). First, 43S complexes comprising 40S subunits, eIF2/GTP/Met-$tRNA^{Met}_i$ ternary complexes (TCs), eIF3, eIF1, and eIF1A attach to the 5′-proximal region of mRNA and then scan along the 5′ untranslated region (5′UTR) to the initiation codon where they stop, forming 48S complexes.

Attachment of 43S complexes to mRNA is mediated by eIFs 4F, 4A, and 4B. eIF4F comprises eIF4E (cap-binding protein), eIF4A (a DEAD-box RNA helicase, whose activity is enhanced by eIF4G and eIF4B), and eIF4G (which binds eIF4E, eIF4A, and also eIF3). eIF4F/4A/4B cooperatively unwind the cap-proximal region of mRNA allowing 43S complexes to bind and likely promote binding via the eIF4G-eIF3 interaction. The molecular mechanism by which mRNA enters the mRNA-binding cleft of the 40S subunit (e.g., by threading through this entire channel starting from its entrance, or by direct placement of the cap-proximal mRNA segment into the mRNA-binding cleft) is unknown.

Ribosomal scanning consists of two linked processes: unwinding of secondary structure in the 5′UTR and ribosomal movement along it. During scanning, 43S complexes must be able to reject potential mismatches between the Met-$tRNA^{Met}_i$ and non- and near-cognate codons, but also to recognize the correct initiation triplet. The key role in ensuring accurate initiation codon selection belongs to eIF1, which enables 43S complexes to discriminate against 48S complex formation on non-AUG triplets and on AUG triplets in suboptimal context (Pestova and Kolupaeva, 2002; Pisarev et al., 2006). eIF1 binds to the interface surface of the 40S subunit between the platform and Met-$tRNA^{Met}_i$ (Lomakin et al., 2003), and it has been suggested that it performs its monitoring function indirectly, by influencing the conformation of ribosomal complexes. Consistently, binding of eIF1 and eIF1A to yeast 40S subunits induces conformational changes that consist of opening of the entry channel "latch" formed between helix (h) 18 in the body and h34 and ribosomal protein (rp) S3 in the neck and establishment of a new head-body connection likely mediated by h16 and rpS3 (Passmore et al., 2007). But what is the role of different factors in ribosomal movement per se? 43S complexes containing TCs, eIF3, eIF1, and eIF1A can bind to the 5′ end of an unstructured 5′UTR and scan to the initiation codon without ATP or factors associated with ATP hydrolysis and RNA unwinding, revealing the intrinsic ability of 43S complexes to move along mRNA (Pestova and Kolupaeva, 2002). Importantly, omission of eIF1A greatly reduces the ability of 43S complexes to form 48S complexes in the absence of eIF4A/4G/4B, and omission of eIF1 almost abrogates it. Although eIF3 is indispensable for 48S complex formation, it is difficult to separate its role in scanning from functions in recruitment of TCs to 40S subunits and initial attachment of 43S complexes. Scanning on 5′UTRs containing even weak internal secondary structure, on the other hand, requires ATP


A
RSW
AS fractionation
(0-40%)
DEAE
(0.1 M KCl)
P-11
(0.3-0.4 M KCl)
Mono S
(300 mM KCl)
Mono Q
(250 mM KCl)
Hydroxyapatite
(300 mM Phosphate buffer)
MW
188
98
62
49
38
28
17
14
6
DHX29
B
DExH
HA2
DUF 1605
1
595
984
1048
1139
1179
1302
1369
motif 1
motif 1a
motif 1b
motif II
motif III
motif IV
motif V
motif VI
Sc prp2 GETGSGKT (23) QPRRVA (40) TDGML (18) DEAH (28) SAT (58) LVFLTGQE (54) TNIAETSLT (38) QRAGRAGR
Hs DHX16 GETGSGKT (22) QPRRVA (39) TDGML (18) DEAH (28) SAT (57) LVFLTGQE (54) TNIAETSPT (38) QRAGRAGR
Hs DHX9 GATGCGKT (25) QPRRIS (40) TVGVL (16) DEIH (28) SAT (108) LVFLPGWN (51) TNIAETSIT (38) QRKGRAGR
Hs DHX29 GETGSGKS (26) QPRRIS (44) TTGVL (18) DEVH (28) SAT (134) LIFLPGLA (51) TNIAETGIT (38) QRQGRAGR
595
629
679
702
734
837
930
977
Sc = Saccharomyces cerevisiae
Hs = Homo sapiens
DHX16 = human prp2 = NP_003578
DHX9 = RNA helicase A = Q08211
prp2 = NP_014408
C
5' G-(CAA)14 - STEM -CAACAACAACCAUGG...[GUS]
ΔG =
-5.5 kCal/mol
-13.1 kCal/mol
-18.9 kCal/mol
-27.6 kCal/mol
No stem
Stem-1
Stem-2
Stem-3
Stem-4
mRNA
40S, eIFs 2/3/1/1A
eIFs 4A/B/F
DHX29
Stem-4 Sequence
48S
AUG
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 C T A G
D
NCF2 mRNA
40S
eIFs 2/3/1/1A
eIFs 4A/B/F
DHX29
Full length
AUG
48S
C T A G 1 2 3 4 5 6
E
CDC25 mRNA
40S, eIFs 2/3/1/1A
eIFs 4A/B/F
DHX29
Full length
AUG
48S
G A T C 1 2 3 4 5
F
5' G-(CAA)14 - STEM -CAACAACAACC-MVHC-STOP...[GUS]
Stem-4
Stem-3
mRNA
40S, 60S, eIFs, eEFs
DHX29
AUG
UGC (Cys)
+16-17 nt from UGC
G A T C 1 2 3 4 5 6
80S
[35S]-MVHC (c.p.m.x10-3)
12
8
4
0
0
4
8
12 Fractions
CAA-Stem3-MVHC mRNA, 40S/60S/eIFs/eEFs
CAA-Stem3-MVHC mRNA, 40S/60S/eIFs/eEFs, DHX29